

## **Appendix for:**

# **Human GBP1 is a microbe-specific gatekeeper of macrophage apoptosis and pyroptosis**

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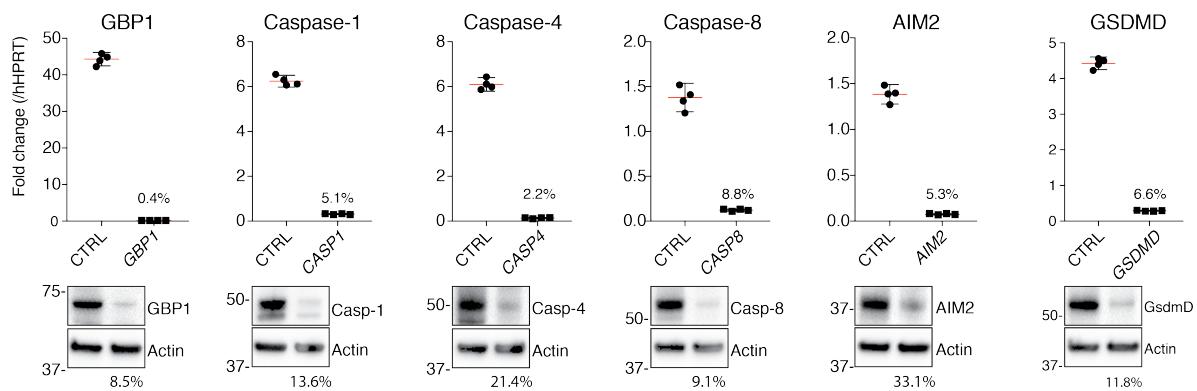
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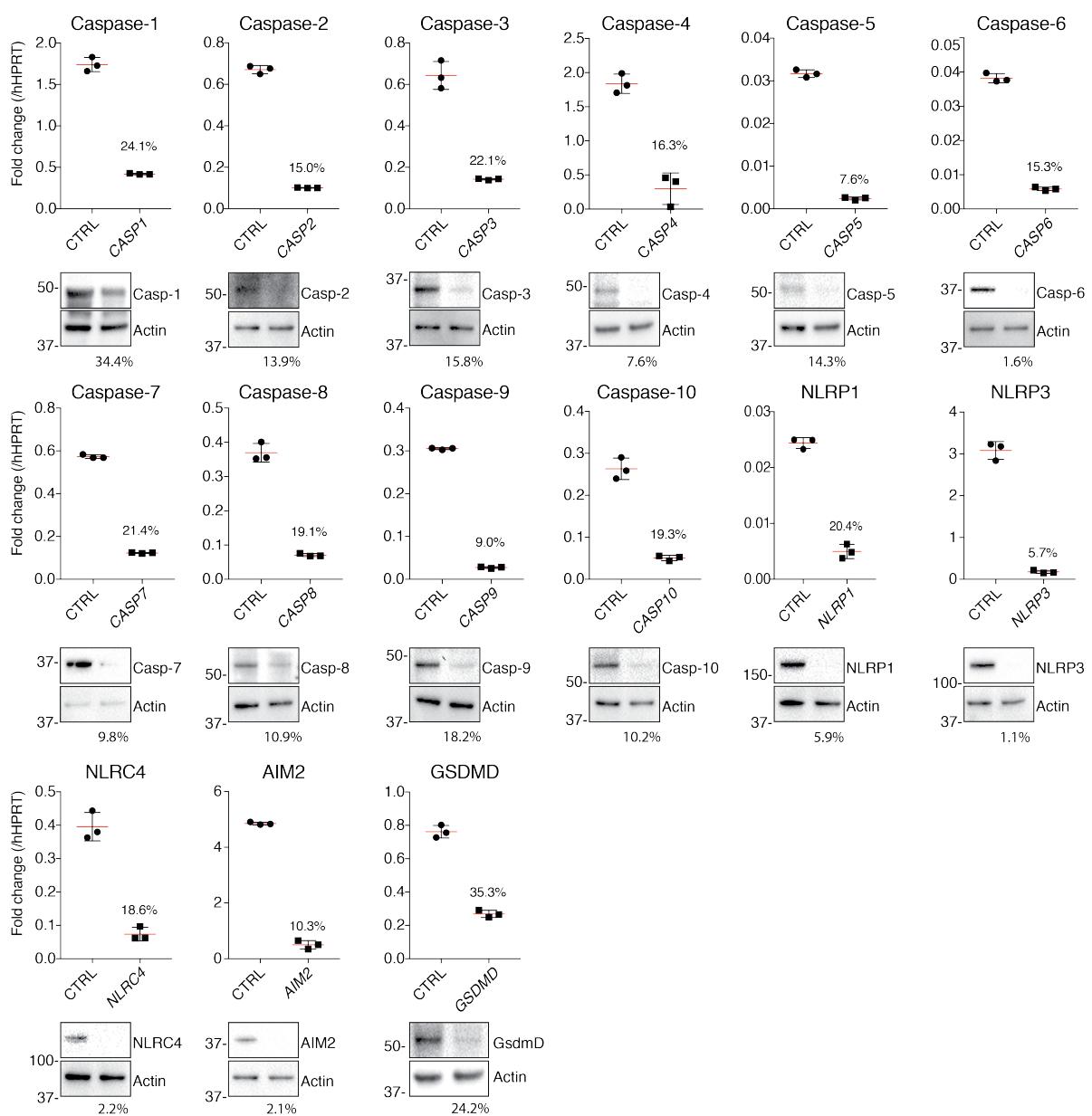
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**A**

### primary monocyte derived macrophages (MDMs)

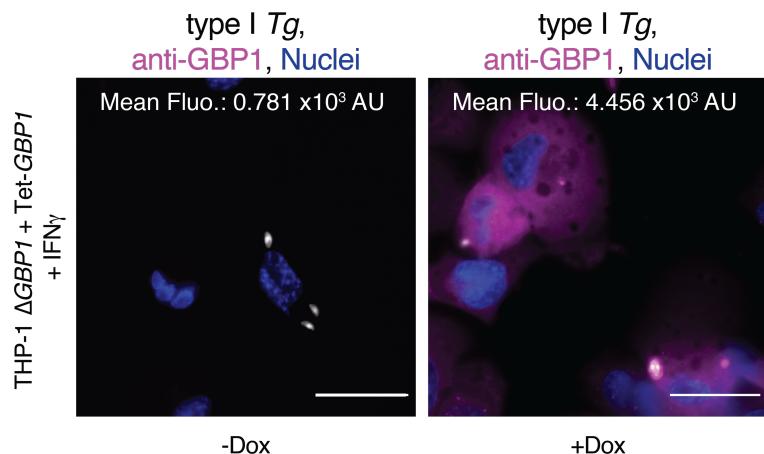
**B**

### differentiated THP-1s



**Appendix Figure S1: Efficiency of siRNA-mediated silencing of expression of genes and protein levels in THP-1 and primary monocyte derived macrophages**

**(A-B)** Graphs showing mRNA levels (normalized to *HPRT1*) and representative immunoblots from primary, IFN $\gamma$ -primed MDMs **(A)** or PMA-differentiated and IFN $\gamma$ -primed THP-1 **(B)**. Percentage mRNA levels or protein intensity as compared to cells transfected with nontargeting control siRNA (CTRL) are indicated. Mean  $\pm$  SEM from n = 3 independent experiments (THP-1) or n = 4 independent experiments (MDMs) plotted.



**Appendix Figure S2: Anti-GBP1 antibody validation for immunofluorescence stainings**

Immunofluorescence staining of IFN $\gamma$ -primed THP-1  $\Delta$ GBP1+Tet-GBP1 cells (all GBPs present but GBP1) infected with type I *Tg* (grey) at 6 hours post infection. Cells were left untreated or pre-treated with Doxycycline (Dox) to induce GBP1 expression and stained with home-made anti-GBP1 antibody (magenta). Mean GBP1 fluorescence signal (Mean Fluo.) is indicated in the images. Scale bar, 40  $\mu$ m.

**Appendix Table S1: Cell lines**

Cell line	Description
A549	Adenocarcinoma alveolar basal epithelial cells
HEK 293T	Human embryonic kidney cells for virus production
HFF	Human foreskin fibroblasts for culturing <i>Toxoplasma gondii</i>
MDMs	Monocyte derived macrophages
THP-1 $\Delta$ ASC	THP-1, knockout for <i>ASC</i>
THP-1 $\Delta$ CASP1	THP-1, knockout for <i>CASP1</i>
THP-1 $\Delta$ CASP4	THP-1, knockout for <i>CASP4</i>
THP-1 $\Delta$ GBP1	THP-1, knockout for <i>GBP1</i>
THP-1 $\Delta$ GBP1+Tet	THP-1, knockout for <i>GBP1</i> , expressing Tet transactivator proteins
THP-1 $\Delta$ GBP1+Tet- <i>GBP1</i>	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible <i>GBP1</i> WT
THP-1 $\Delta$ GBP1+Tet- <i>GBP1</i> $\Delta$ 589-592	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible <i>GBP1</i> $\Delta$ 589-592 = CaaX box deletion
THP-1 $\Delta$ GBP1+Tet- <i>GBP1</i> C589A	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible <i>GBP1</i> C589A = no lipidation of CaaX box
THP-1 $\Delta$ GBP1+Tet- <i>GBP1</i> K382R	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible <i>GBP1</i> K382R = mutated ubiquitination site
THP-1 $\Delta$ GBP1+Tet- <i>GBP1</i> K51A	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible <i>GBP1</i> K51A = GTPase dead
THP-1 $\Delta$ GBP1+Tet- <i>GBP1</i> RK227/228EE	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible <i>GBP1</i> RK227/228EE = constitutively dimeric
THP-1 $\Delta$ GBP1+Tet-mCH- <i>GBP1</i>	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible mCherry-tagged <i>GBP1</i> WT
THP-1 $\Delta$ GBP1 + Tet-mCH- <i>GBP1</i> $\Delta$ 589-592	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible mCherry-tagged <i>GBP1</i> $\Delta$ 589-592 = CaaX box deletion
THP-1 $\Delta$ GBP1+Tet-mCH- <i>GBP1</i> + YFP-CASP4	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible mCherry-tagged <i>GBP1</i> WT and YFP-tagged caspase-4

THP-1 $\Delta$ GBP1+Tet-mCH- <i>GBP1</i> + YFP-CASP4 C258S	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible mCherry-tagged GBP1 WT and YFP-tagged caspase-4 C258S = catalytically inactive
THP-1 $\Delta$ GBP1+Tet-mCH- <i>GBP1</i> C589A	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible mCherry-tagged GBP1 C589A = no lipidation of CaaX box
THP-1 $\Delta$ GBP1+Tet-mCH- <i>GBP1</i> K51A	THP-1, knockout for <i>GBP1</i> , expressing Doxycycline-inducible mCherry-tagged GBP1 K51A = GTPase dead
THP-1 sh <i>GSDMD</i>	THP-1, stable knockdown of <i>GSDMD</i>
THP-1 WT	THP-1 monocytes, differentiated to macrophage-like cells with PMA

**Appendix Table S2: Antibodies**

Target	Manufacturer	Cat number
GBP1	homemade	-
Caspase-1	Adipogen	AG-20B-0048
Caspase-2	CST	#2224
Caspase-3	CST	#9661
Caspase-4	CST	#4450
Caspase-5	CST	#46680
Caspase-6	CST	#9762
Caspase-7	CST	#12827
Caspase-8	CST	#9746
Caspase-9	CST	#9508
Caspase-10	Genetex	GTX113148
Gasdermin D	CST	#96458
PARP	CST	#9542
NLRP1 (= NALP1)	CST	#4990
NLRP3	CST	#13158
NLRC4	CST	#12421
ASC	Adipogen	AG-25B-0006
AIM2	CST	#12948
<i>Salmonella</i> LPS	Abcam	ab8271
YFP	Abcam	ab6556

**Appendix Table S3: Primers for qRT-PCR**

<b>Gene</b>		<b>Sequence 5'-3'</b>
GBP1	fwd	TATTGCCCACTATGAACAGCAGAT
	rev	TAGCTGGGCCGCTAACCTCC
GBP2	fwd	AATTAGGGGCCAGTTGGAAG
	rev	AAGAGACGGTAACCTCCTGGT
GBP3	fwd	GAATAAGGGCTTCTCTGGC
	rev	AGTGTCAAGCAGGACTAAGGTG
GBP4	fwd	TAAGCGGCTTCAGAGCACC
	rev	GACCTCGTTGCCTTAACCTCC
GBP5	fwd	CCTGATGATGAGCTAGAGCCTG
	rev	GCACCAGGTTCTTAGACGAGA
GBP6	fwd	TGCACCATCCCATTGTGGAA
	rev	TGCCAACCTAGAAGAGCCTGC
GBP7	fwd	GAGTTAAGGCAGACGAGGTCC
	rev	TTCAGCTGCCTCCTTCTTAGC
ASC	fwd	ATCCAGGCCCTCCTCAG
	rev	AGAGCTTCCGCATCTTGCTT
AIM2	fwd	ACAGGCCTGGATAACATCACT
	rev	ACCGCCCCAGCATTGAAAT
NLRP1	fwd	GGACCAGTATCGAGAGCAGC
	rev	GAGGTGAGGATGGGTCTCCT
NLRP3	fwd	TCCTGGCTGTAACATTGGAG
	rev	TGCAAGATCCTGACAACATGC

NLRC4	fwd	CGGCAGAGGGTTCTTTCCT
	rev	ATATCCCCACCTCAGCAGT
GsdmD	fwd	TAGTCCGGAGAGTGGTCCAG
	rev	ACCATGAGCTTGAGGGCTTC
Casp1	fwd	AATGGACAAGTCAAGCCGCA
	rev	AGTCATGTCCGAAGCAGTGAG
Casp2	fwd	GTTACCTGCACACCGAGTCA
	rev	TCAGTCTCATCTCCACGGCA
Casp3	fwd	CTCGGTCTGGTACAGATGTCGA
	rev	CATGGCTCAGAAGCACACAAAC
Casp4	fwd	CTGTTCCCTATGGCAGAAGGC
	rev	TCTGCCATGACCCGAACCTT
Casp5	fwd	GCAAGGAATGGGGCTCACTA
	rev	CGTGCTGTCAGAGGACTTGT
Casp6	fwd	AAATGGACCACAGGAGGAGAG
	rev	CTGAAAACCTGCGGGTAAGA
Casp7	fwd	ACATGAATTGAAAAGCTGGG
	rev	CAGGCAGGCATTGTATGGTC
Casp8	fwd	GGTCACTTGAACCTTGGGAA
	rev	AGGCCAGATCTCACTGTC
Casp9	fwd	GTGGACATTGGTTCTGGAGGAT
	rev	CGCAACTTCTCACAGTCGATG
Casp10	fwd	ATCCTTCGGCATGTGGAGG
	rev	TAGTGTGAAAGCAGGCTGGG

HPRT	fwd	ACCAGTCAACAGGGGACATAA
	rev	CTTCGTGGGTCCCTTCACC

**Appendix Table S4: Primers for cloning**

Name	Sequence 5'-3'	Purpose
LSC-5_repair_fwd	CGGGAGTATCCG	Deleting sgRNA cassette from pLSC-
LSC-5_repair_rev	AATTGGATACTCCCGGTAC	
LSC-5_repair_seq	GGGACAGCAGAGATCCAGTT	5 + Sequencing
CMV_fwd	CGCAAATGGGCGGTAGCGTG	Sequencing
EFS_fwd	TGAACGTTCTTTTCGCAAC	Tet vectors
EFS_Seq_rev	GTTGCGAAAAAGAACGTTCA	
mCH_seq-rev	CGAAGTTCATCACGCGCTC	
Tet-vector_Seq-fwd	TCCACGCTGTTTGACCTC	
Tet_hGBP1_Seq_rev1	CTTAGTGTGAGACTGCACCGTGG	
Tet-Off_BB-fwd	GACTTGAGCTGTCGCCGCCAGGCCATAAAGCGGCCGAC TCTAGA	Cloning Tet-ctrl vector by Gibson assembly
Tet-ON_BB-rev	TATGACTTGCTCTGTCCAGTCTAGACATCCGTCGACTGCA GAATTCGAAGC	
BB_Tet-ON-fwd	AGCTCAAGCTTCGAATTCTGCAGTCGACGGATGTCTAGACTG GACAAGAGC	
P2A_Tet-ON-rev	CAGCAGAGAGAAGTTGTCGCCGGATCCCCGGGGAGCAT GT	

Tet-ON_P2A-fwd	GACGATTTGACCTTGACATGCTCCCCGGGGATCCGGCGCA ACAAAC	
Tet-OFF_P2A-rev	GTAAATCACTTACTTTATCTAATCTAGACGGTCCAGGATT CTCTTCGAC	
P2A_Tet-OFF-fwd	GCCGGAGATGTCGAAGAGAACCTGGACCGTAGATTAGAT AAAAGTAAAGTGATTAACAGCG	
BB_Tet-OFF-rev	GGCTGATTATGATCTAGAGTCGCGGCCGCTTATGGCCTGGG GCG	
bsd_BB-fwd	CTGCCCTCTGGTTATGTGTGGAGGGCTAAAGCGGCCGAC TCTAGA	
T2A_BB_rev	CAGCAGAGAGAAGTTGTTGCGCCGGATCCTGGCCTGGGCG GC	
BB_T2A_fwd	TTGGACTTGAGCTGTCGCCGCCAGGCCAGGATCCGGCGCA ACAAAC	
bsd_T2A_rev	AATGAGGGTGGATTCTTCTTGAGACAAAGGCGGTCCAGGATT CTCTTCGAC	
T2A_bsd_fwd	GCCGGAGATGTCGAAGAGAACCTGGACCGCCTTGTCTCAA GAAGAATCCACC	
BB_bsd_rev	GGCTGATTATGATCTAGAGTCGCGGCCGCTTAGCCCTCCA CACATAACC	
bsd_Seq_fwd	GATCGGAAATGAGAACAGGG	
BB_insert-fwd	TTGCCGCCAGAACACAGGACCGGTGCCACCATGTCTAGACTG GACAAGAGC	
BB_insert-rev	ATCCAGAGGTTGATTGTCGACTTAACCGTTCAGCCCTCCA CACATAAC	
Insert_BB-fwd	CTGCCCTCTGGTTATGTGTGGAGGGCTAAACCGCTTAAGTC GACAATCAACCTC	

Insert_BB-rev	TATGACTTTGCTCTTGTCCAGTCTAGACATGGTGGCACCGGT  CCTG	
BB_Zeo-fwd	TTGCCGCCAGAACACAGGACCGGTGCCACCATGGCCAAGTTG  ACCAGTG	Cloning  pLenti-Tet  vector
BB_Zeo-rev	ATCCAGAGGTTGATTGTCGACTTAACCGTTCAGTCCTGCTC  CTCGG	
Zeo_BB-fwd	GTGCACCTCGTGGCGAGGAGCAGGACTGAACCGTTAAGTC  GACAATCAACCTC	
Zeo_BB-rev	CACCGGAACGGCACTGGTCAACTTGGCCATGGTGGCACCGGT  CCTG	
BB_Tet-Prom-fwd	TCAAAATTTCGGGTTTATTACAGGGACAGCTCGAGTCCGGA  TCTCGAC	
BB_Tet-Prom-rev	GGTACCTTAATTAACCAAACGGATCTCTGCTAGTTGGTT  TGTCCAAACTCATC	
Tet-Prom_BB_fwd	CATTGATGAGTTGGACAAACCACAACTAGCAGAGATCCAGT  TTGGTTAATTAAGG	
Tet_Prom_BB-rev	GACGTGCCCGGGTCGAGATCCGACTCGAGCTGCCGTAA  TAAACCCG	
BB_Tet-Prom-fwd2	TTTGGTTAATTAAGGTACCGGGAGTATCCGCTCGAGTCCGGA  TCTCGAC	
BB_Tet-Prom-rev2	CCACTCCTTCAAGACCTAGCTAGCGAATTCTAGTTGGTT  TGTCCAAACTCATC	
MCS-fwd	GATCCCTGATCGATCGGCCG	
MCS-rev	AATTCGGCCGATCGATCAGG	
hGBP1_K51A_fwd	GCCTCTACCGCACAGGCGCATCCTACCTGATGAACA	Site directed

hGBP1_K51A_rev	TGTTCATCAGGTAGGATGCGCCTGTGCGGTAGAGGC	mutagenesis of GBP1
hGBP1_RK227-228EE_fwd	GCATTTTTCTTGGAAAGAATTCCCTCGATAACAGAGTCTGGG CAGGTTA	
hGBP1_RK227-228EE_rev	TAACCTGCCAGACTCTGTATCGAGGAATTCTCCCAAAGAA AAAATGC	
hGBP1_K382R_fwd	GCCGCTAACCTCCTTGAAATAGATGGTCCACATCT	
hGBP1_K382R_rev	AGATGTGGACCATCTATTCAAAGGGAGTTAGCGGC	
BB_hGBP1_fwd	AATTAGCGCTACCGGTGCGGCCGCACCATGGCATCAGAGTC CACATGACAGG	Gibson assembly of GBP1 into pLenti-Tet and deleting the C-terminus/ mutating C589
BB_hGBP1-wt_rev	CTAGCGAATT CGGCCGATCGATCAGGGATCTAGCTTATGGT ACATGCCTTCGTCGTC	
BB_hGBP1-delta589-592_rev	CTAGCGAATT CGGCCGATCGATCAGGGATCTTAGCTTATGCCTTCG TCGTCTCATTTCGTC	
BB_hGBP1-C589A_rev	CTAGCGAATT CGGCCGATCGATCAGGGATCTAGCTTATGGT AGCTGCCTTCGTCGTC	
hGBP1_mCH_rev	TGGGCCTGTCATGTGGATCTCTGATGCCATCTACTTGTACAG CTCGTCCATGCC	Adding mCH to the GBP1 N-terminus during Gibson assembly
mCH_hGBP1_fwd	TCCACCGGCCGATGGACGAGCTGTACAAGATGGCATCAGAG ATCCACATGACAGG	
Tet_mCH_Gibson_fwd	AATTAGCGCTACCGGTGCGGCCGCACCATGGTGAGCAAGGGC GAGG	

Tet_hGBP1_S eq_fwd1	CCACGGTGCAGTCTCACACTAAAG	Sequencing Tet-GBP1 vectors
Tet_hGBP1_S eq_fwd2	TCAATGCCATCAGCAGTGGG	
Tet_hGBP1_S eq_fwd3	GAAGCATCATCAGATCGTTGC	
ACTB-ctrl_fwd	GTGCTATCCCTGTACGCCTC	PCR ctrl for genotyping
ACTB-ctrl_rev	GCAGCTCGTAGCTCTTCTCC	
GBP1_cDNA_f wd	GCCTGGACATGGCATCAGAG	Amplifying GBP ORFs for sequencing
GBP1_cDNA_r ev	GGTGACAGGAAGGCTCTGG	
GBP2_cDNA_f wd	CCTGGACATGGCTCCAGAGA	and subcloning
GBP2_cDNA_r ev	GCTGGACAGGCAAATTTGCTC	
GBP3_cDNA_f wd	CAGACAAGAGAACAAATGCCCTGG	
GBP3_cDNA_r ev	GCTCTGTTGTTAGATCTTAGCTTATGCG	
GBP4_cDNA_f wd	GAGGACAGAGCAATGGGTGAG	
GBP4_cDNA_r ev	CAGGCTCTTAAATACGTGAGCCAAG	
GBP5_cDNA_f wd	CATCCTAGACATGGCTTAGAGATCCAC	

GBP5_cDNA_rev	CTCCCCATTTAGCACTTAGAGTAAACACATG	
GBP1_gRNA1_fwd	CACCGAGGCTTCTCTCTGGGCTCCA	Cloning GBP1 gRNA1 plasmid
GBP1_gRNA1_rev	AAACTGGAGCCCAGAGAGAACGCCTC	
GBP1_gRNA2_fwd	CACCGGGCTTCAGCAAAAATGTTGC	Cloning GBP1 gRNA2 plasmid
GBP1_gRNA2_rev	AAACGCAACATTGGCTGAAGCCC	
GBP1-KO check1_fwd	CATGGCATTATTATGTTGAGGTGC	gDNA
GBP1-KO check1_rev	ATTTGTCCTCCCTTATGCATCTGT	Sequence analysis for CRISPR result
GBP1-KO check2_fwd	GATTGAAGGTGAGGAGTGAGTTAAG	
GBP1-KO check2_rev	AGTACAAGTAAGCAAGCAGGGTCCT	
CASP4-EcoRI-fwd	atctcgagctcaagcttcgATGGCAGAAGGCAACCACA	Cloning pMX-YFP-
CASP4-EcoRI-rvs	ctcgaggcctgcaggaattcTCAATTGCCAGGAAAGAGGTA	Caspase-4
CASP4-C258S-fwd	catcattgtccaggccTCCagaggtgcaaacc	Site- directed mutagenesis
CASP4-C258S-rvs	ggtttgcacctctGGAggcctggacaatgatg	

